Vol. 157, No. 2 October, 1979

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

Reference: *Biol. Bull.*, 157: 221–233. (October, 1979)

SALT AND WATER BALANCE IN TWO MARINE SPIDER CRABS, *LIBINIA EMARGINATA* AND *PUGETTIA PRODUCTA*. I. URINE PRODUCTION AND MAGNESIUM REGULATION

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The excretory organs of decapod crustaceans function in magnesium regulation (Robertson, 1957; Lockwood, 1962; Potts and Parry, 1964) and to a lesser extent, in nitrogen excretion (Delaunay, 1931; Binns and Peterson, 1969). Magnesium regulation has been observed in decapods from a variety of habitats (Robertson, 1939, 1949, 1953; Webb, 1940), the usual pattern being a lowered blood concentration and an elevated urine concentration, with respect to the medium. This phenomenon has been investigated extensively in two osmoregulating crabs, *Pachygrapsus crassipes* (Prosser, Green and Chow, 1955; Gross and Marshall, 1960; Gross and Capen, 1966) and *Carcinus maenas* (Riegel and Lockwood, 1961; Lockwood and Riegel, 1969), but little is known about magnesium regulation in osmoconforming crabs.

A problem in the study of magnesium regulation, and other aspects of salt and water balance, is that of making accurate estimates of urine production rates. Cannulation is usually not feasible because of the geometry and delicacy of the excretory duct. Many methods have been used, perhaps the best known being nephropore-occlusion, but only a few estimates have been made for an extended period of time by the continuous collection of urine: *Procambarus clarkii* (Kamemoto and Ono, 1968; Ono and Kamemoto, 1969); *Paranephrops zealandicus* (Wong and Freeman, 1976); *Cancer magister* (Holliday, 1977); *Callinectes sapidus* (Cameron and Batterton, 1978). A technique for the continuous collection of urine has been used in the present study.

Materials and Methods

Specimens of the osmoconforming crab, *Pugettia producta*, were collected near the Bodega Marine Laboratory, Bodega Bay, California, and maintained at 10 to

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Library of Congress Card No. A38-518
(ISSN 0006-3185)

12° C, either in running sea water (SW) at the Bodega Marine Laboratory, or in filtered SW (about one crab per 4 liters) at the University of California, Berkeley, California. Intermolt crabs were used except where otherwise noted.

Estimates of urine production were made by continuous collection of urine. A polyester resin cast of the region surrounding the two nephropores was made in order to position two polyethelene tubes. In making the cast, the crab was clamped in a supine position and the periopods secured with rubber bands. The ventral surface was dried with compressed air and those portions of the third maxillipeds distal to the ischia were cut off. The region surrounding the nephropores was lightly swabbed with vaseline which acted as a mold releasing agent. A transverse dam of plasticine, placed distal to the third maxillipeds, prevented the casting material from flowing into the mouth parts. A freshly mixed polyester resin, such as "G-R-R-R-I-P" (Idaho Chemical Industries, Inc.) was applied so that it extended posteriad to the plasticine dam, anteriad to the antennules, and laterad to about 5 mm beyond the nephropores. After 15 min, the cast was removed and the crab returned to SW. This procedure was carried out at least 24 hr before the start of an experiment.

The impressions of the opercula that cover the openings of the nephropores could clearly be seen in the cast. Each impression was used to center a hole of 0.063 inch (1.60 mm) diameter. A drill, larger by about 20% than the diameter of the opercula, was used to countersink the first pair of holes to a depth slightly greater than the diameter of the opercula. This allowed sufficient room for the opercula to open. Polyethelene tubing (P.E.Intramedic 190, Clay Adams) was pressed into the holes in the cast. Silicone grease was carefully applied around the periphery of the cast and between the two holes on its inner face. The crab was replaced in the clamp, the periopods secured with rubber bands, and the region surrounding the nephropores dried with compressed air. The cast, with the attached tubing, was positioned and pressed onto the crab. In most cases it snapped into position and was firmly held in place by a rubber band.

The crab was then suspended in an aquarium so that the top of the carapace was just submerged. The seal between the cast and the animal was checked by blowing into the tubes and watching for air bubbles. The tubes were led over the lip of the aquarium, about 3 to 5 cm above the nephropores, and fed into two vials, positioned at about the same level as the nephropores. The urine was collected under mineral oil.

Urine production was also estimated by blocking the nephropores with Eastman 910 cement (Armstrong Cork Co.) and measuring the change in weight. The Eastman 910 was allowed to set for 10 min before the crabs were returned to SW. During this period, excess water was removed from the branchial chambers, the animals were dried for one minute with compressed air, and weighed.

The concentrations of sodium, magnesium, calcium, potassium, chloride, and ninhydrin positive substances (NPS), and osmotic pressure, in blood, urine and SW were measured. Blood samples were withdrawn by puncturing an arthrodial membrane at a leg base with a drawn-out Pasteur pipet. Samples were centrifuged under mineral oil for 10 min in either an International Clinical Centrifuge at about 6000 rpm at room temperature or a Sorval model RC2-B, at 10,000 rpm at 1° C, the latter being used in the preparation of samples for osmotic pressure

determinations. Urine samples were also collected at the nephropore. A small hook, guided with the aid of a dissecting scope, was used to lift an operculum and the urine was collected in a drawn-out Pasteur pipet. Samples of plasma, urine and medium were stored under mineral oil in polystyrene vials.

Concentrations of cations were measured with a Perkin-Elmer model 290 atomic absorption spectrophotometer. Concentrations of chloride were measured on a Buchler-Cotlove chloridometer. NPS were determined by the method of Fowden (1951). Plasma was deproteinized by the addition of an equal volume of 10% trichloroacetic acid. For uniformity, urine received the same treatment. Samples were read on a Klett colorimeter against glycine standards. Measurements of osmotic pressure were made with an Advanced Instruments "Osmette" osmometer. Measurements of electrical potential difference across the body wall were made with an Analog Devices model 40J operational amplifier (input impedance, 10^{11} ohms) as a preamplifier and voltages were read on a Tektronix oscilloscope. Chlorided silver wires in 3 M KCl were used to make the electrical connections to 3 M KCl-agar bridges. One bridge served as the reference electrode while the other was placed in a hole in the top of the carapace, which remained out of water.

Results

Urine production rates estimated by continuous collection

Figure 1 shows the results of an experiment where urine was collected over a 24-hr period. The sum of the cumulative volume of urine released from both nephropores has been arbitrarily fitted with a fifth order polynomial. The urine production rate, the first derivative of this polynomial, is also included. The rate of urine production was usually greater during the first 12-hr period than during subsequent collecting periods. The rates which will be reported were obtained after a 12-hr lapse from the start of collection. Urine release is intermittent, the interval between successive releases can vary greatly, and urine is usually released simultaneously from both nephropores.

Intermolt specimens of *Pugettia producta* (average weight, 101 g) in 100% SW produced urine at 6.40 ± 3.08 (22) % body weight (bw)/day, mean $\pm$ SD (N). Postmolt crabs (73 g) produced urine at 2.89 ± 3.68 (5) % bw/day and premolt crabs (65 g) produced urine at 29.5 ± 3.98 (3) % bw/day. A single classification analysis of variance indicates that there are significant differences among these means ($P < 0.01$) and *a priori* tests indicate that there are differences between the rates for intermolt and premolt crabs ($P < 0.01$), and intermolt and postmolt crabs ($P < 0.05$). The cause for these differences is not known.

Urine production rates for 10 crabs (114 g) were compared on two successive days. The average rate on the first day was 5.06 ± 3.68% bw/day; on the second day it was 5.48 ± 3.27 % bw/day. These means are not significantly different (paired *t*-test). For one crab, urine production was measured for a 2-week period. During this time, the rate varied from 2 to 6% bw/day, the average being 3.02% bw/day. There was no apparent pattern to the daily fluctuations, and it is possible that the changes could be accounted for by changes in the volume of urine held in the bladders, which in *Pugettia* can exceed 5% bw (Cornell, 1976).

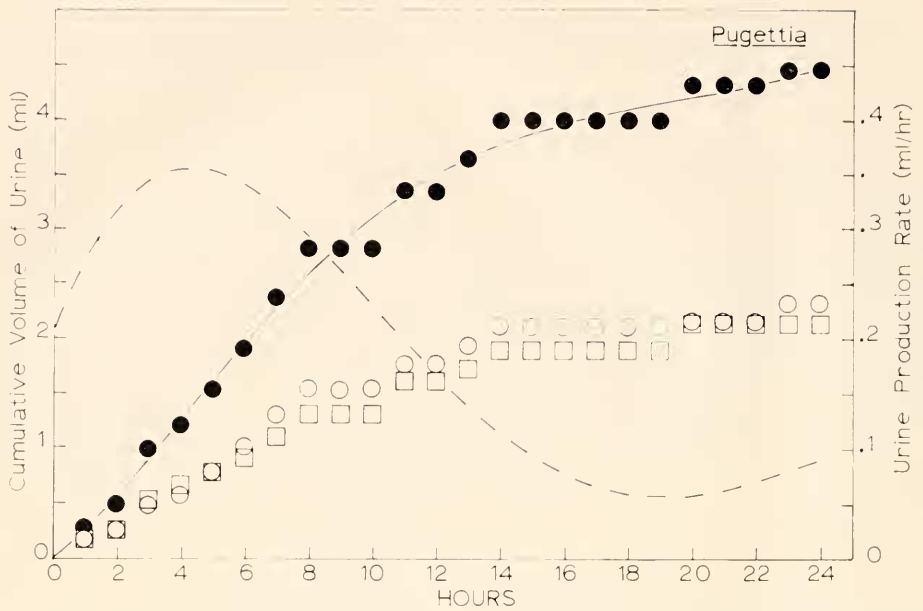

FIGURE 1. Urine production in a 46 g specimen of *Pugettia producta* from the time of placement of the polyester resin cast until the 24th hr. The cumulative volume of urine produced from the left and right nephropores is indicated by the square boxes and open circles, respectively, and can be read on the left ordinate. The sum of the cumulative volume of urine from both nephropores, indicated by solid circles, has been arbitrarily fitted with a fifth order polynomial, indicated by the solid curve. The first derivative of this polynomial, or urine production rate, is indicated by the dashed line and can be read on the right ordinate.

Urine production estimated from weight gain

Intermolt specimens of nephropore-occluded *Pugettia* (43.54 g) gained 3.38 ± 2.10 (12) % bw after 24 hr, while a control group (46.10 g) gained 0.43 ± 0.88 (9) % bw in the same period. Four crabs with blocked nephropores gained an additional 1.0% between 24 and 48 hr. These animals were markedly swollen, the posterior region of the carapace being lifted away from the abdomen. The urine production rate for intermolt crabs determined by weight gain is significantly less than that determined by continuous collection ($P < 0.005$, t-test), suggesting that the rates estimated by weight gain are under-estimates of the true rate, and that significant back pressures can occur.

Some major constituents of blood and urine

Measurements of some major constituents of blood, urine and medium for crabs in 100% SW are presented in Table I. An approximate statistical test (Sokal and Rohlf, 1969, p. 372), analogous to a single classification analysis of variance, was used to test for equality among means since these data are heteroscedastic.

The concentrations of sodium and chloride in the blood were both 98% of

their respective concentrations in the medium. In the urine, the concentration of chloride was about equal to, while sodium was 96% of, the concentration in the medium. The concentrations of magnesium, calcium and potassium in the blood were 88, 118, and 105% of their respective concentrations in the medium. In the urine, these same ions were 135, 124, and 114% of the medium, respectively. NPS were about 10 times more concentrated in the blood than in the urine. The osmotic pressure of the blood was about 2 mosM greater than that of the medium and the urine was isosmotic to the blood, but not statistically different from the medium.

The concentrations of inorganic ions were more variable in the urine than in the blood; in particular, magnesium was the most variable, as judged by the ratio of the standard deviation to the mean. Magnesium concentrations ranging from 48 to 128 mM were observed in the urine. Table II shows two correlation matrices, one each for the blood and the urine. In each matrix, a rank correlation coefficient has been computed for each inorganic ion with every other inorganic ion. There is a significant negative correlation between sodium and magnesium in the urine. There are also significant positive correlations between magnesium and calcium and between magnesium and potassium in the urine.

In the blood, magnesium concentrations were also relatively more variable than the other ions which were measured. A significant negative correlation between magnesium and calcium was found, the reverse of the condition in the urine. Significant positive correlations between magnesium and chloride, and between sodium and potassium, were also found.

TABLE I

Concentrations of some major constituents of the blood and urine of specimens of Pugettia producta *in sea water. Relative concentrations are expressed as a percentage of the medium. Absolute concentrations of inorganic ions, ninhydrin positive substances (NPS) and osmotic pressure (OP) are expressed in mM, mM glycine, and mosM, respectively. F(df) indicates the F ratio of an approximate test with the calculated degrees of freedom. One and two asterisks denote $P < 0.05$ and $P < 0.01$, respectively.*

Solute	Fluid Relative concentration Absolute concentration, mean ± sd (N)			F (df)
	Blood	Urine	Medium	
Cl^-	98.0% 524 ± 12.9 (13)	99.4% 532 ± 27.9 (14)	100% 535 ± 2.73 (6)	4.24* (2, 18)
Na^+	97.8% 450 ± 16.0 (23)	96.1% 442 ± 22.1 (37)	100% 460 ± 5.74 (6)	9.60** (2, 30)
Mg^{2+}	88.3% 46.1 ± 2.48 (23)	135% 70.5 ± 19.2 (37)	100% 52.2 ± 0.65 (6)	75.7** (2, 24)
Ca^{2+}	118% 11.8 ± 0.52 (13)	124% 12.4 ± 1.11 (22)	100% 10.0 ± 0.17 (6)	95.6** (2, 43)
K^+	105% 10.2 ± 0.38 (13)	114% 11.1 ± 1.13 (22)	100% 9.74 ± 0.19 (6)	11.5** (2, 21)
NPS	— 3.72 ± 2.00 (14)	— 0.39 ± 0.12 (5)	— —	38.4** (1, 13)
OP	100.2% 1016 ± 2.47 (10)	100.2% 1016 ± 3.77 (10)	100% 1014 ± 1.19 (10)	3.69* (2, 15)

TABLE II

Correlation matrices (Spearman's rank correlation coefficient, N) for inorganic ions in the blood and urine of Pugettia producta *in sea water. One and two asterisks denote $P < 0.05$ and $P < 0.01$, respectively.*

Blood

Urine	Cl⁻	Na⁺	Mg²⁺	Ca²⁺	K⁺	Blood
Cl⁻		0.38, 13	0.55, 13*	−0.41 13	0.30, 13	Cl⁻
Na⁺	−0.03, 14		−0.02, 23	−0.36, 13	0.48, 13*	Na⁺
Mg²⁺	0.19, 14	−0.40, 37*		−0.64, 13*	−0.13, 13	Mg²⁺
Ca²⁺	0.20, 14	−0.19, 16	0.81, 16**		−0.23, 13	Ca²⁺
K⁺	−0.40, 14	−0.01, 16	0.50, 16*	0.41, 16		K⁺
	Cl⁻	Na⁺	Mg²⁺	Ca²⁺	K⁺	

Urine

TABLE III

Magnesium and sodium concentrations in the blood of normal and nephropore-occluded specimens of Pugettia producta *in sea water. See text for statistical tests.*

Ion	Time					
	0 hr		24 hr		48 hr	
	Normal	Occluded	Normal	Occluded	Normal	Occluded
Mg^{2+} (mM) sd, N	47.1 2.05, 7	47.5 2.48, 10	46.8 1.01, 7	48.5 4.33, 10	46.1 2.44, 7	51.4 3.87, 10
Na^{+} (mM) sd, N	448 14.8, 7	454 20.1, 10	450 4.5, 7	462 28.9, 10	446 16.8, 7	473 23.9, 10

The effects of nephropore-occlusion on magnesium regulation

Of the inorganic ions studied, magnesium represents the clearest example of an ion which is regulated by the excretory system. Its concentration in the blood, urine, and medium suggest that it is continuously diffusing down the concentration gradient from medium to blood, and that this gradient is maintained by its active removal by the excretory system. Estimates of the electrical potential difference across the body wall of three crabs indicate a difference of less than 0.1 mV. Thus, the driving force for the diffusion of magnesium from the medium to the blood must be the difference in chemical potential.

Blocking the nephropores should stop the removal of magnesium from the blood, and thus the magnesium level in the blood should rise, reaching equilibrium with the magnesium in the medium. The results of such an experiment appear in Table III. The magnesium concentration in the blood of the experimental animals with blocked nephropores increased; after 48 hr the ratio of the magnesium concentration in experimental crabs to that in control crabs was 1.12. There was also an increase in sodium concentration in the blood of experimental crabs, the above ratio being 1.06. This may indicate that sodium is also normally regulated below its equilibrium concentration. The sodium and magnesium data were separately analyzed by two-way analysis of variance after randomly removing the data for three crabs in the experimental group in order to facilitate the calculations. These analyses indicate that there are significant increases in sodium and magnesium concentrations in the blood of nephropore-occluded crabs ($P < 0.01$ for both ions in the sub-groups Normal and Occluded; for both ions, the F ratios were not significant in the sub-group Time, and for the interaction between sub-groups Normal and Occluded × Time).

In *Pachygrapsus crassipes,* Gross and Capen (1966) demonstrated that the magnesium concentration in the urine is a direct function of the time the urine is held in the bladder. This does not seem to be the case in *Pugettia.* When, after 86 hr, the magnesium concentration in the urine of nephropore-occluded and control crabs were compared, the magnesium concentration in the control crabs, 81.9 ± 26.8 (7) mM, was greater than that in the experimental crabs, 58.4 ± 9.93 (9) mM. This unexpected result seems to have been caused by chance, since the

concentration in the control group was greater than expected. Also, since one of the crabs in the experimental group had died, the remaining animals may not have been in good condition and the transport of magnesium could have been reduced. The experiment was repeated using a paired design and a shorter period of time to minimize the effects of individual variation and nephropore-occlusion. No differences were found in 10 crabs between the magnesium concentrations at 0 hr, 65.3 ± 20.7 mM, and 24 hr, 64.2 ± 18.3 mM, after nephropore-occlusion. These experiments suggest that the bladders of *Pugettia* do not secrete magnesium into the urine.

Discussion

Decapod crustaceans have considerable ability to regulate their internal ionic compositions (Robertson, 1949, 1953). This appears to be independent of the ability to osmoregulate and has been defined by Robertson (1949, p. 182) as the "maintenance in a body fluid of concentrations of ions differing from those of a passive equilibrium with the external medium." Since the blood of decapods contains considerable amounts of non-diffusible, negatively charged protein, the internal/external concentration ratio of an ion can differ passively from unity. This ratio is expected to be less than 1.0 for the anions and greater than 1.0 for the cations. Robertson (1953) found, using a dialysis technique, that it was often 1.03 for the cations, except for calcium for which it was estimated that up to 20% was complexed with proteins. Greenaway (1976) has confirmed these results for calcium.

The present data suggest that *Pugettia producta* hypo-regulates magnesium and sodium; potassium is probably slightly hyper-regulated, while chloride is probably very close to its equilibrium concentration. From these data it is not possible to determine if calcium is regulated, since an unknown amount is complexed with proteins. The comparison of the concentrations of various ions in the blood of *Pugettia* with those of other decapod crustaceans, tabulated by Prosser (1973), indicates that the pattern of regulation in *Pugettia* is similar to that in other marine decapods.

Expressed as mM glycine, the blood and urine of *Pugettia* contain 3.7 and 0.39 mM NPS, respectively. It was the usual policy to use crabs which had not been fed for a week. Thus, NPS in the blood of *Pugettia* fresh from the field could be different. A comparison of these values with those reported for *Carcinus maenas* (Binns, 1969b; Evans, 1972) indicates that the blood concentrations are similar in both crabs when differences in technique are accounted for (see Evans, 1972). The concentration of NPS in the urine of *Pugettia* appears to be about half of that in *Carcinus*, but this can be quite variable and may not represent a true difference. The antennal gland of crustaceans is not a major route for nitrogen loss. Binns and Peterson (1969) estimate that in the spiny lobster *Jasus edwardsi*, 90% of all the soluble nitrogen is excreted extra-renally.

The urine production rate for intermolt specimens of *Pugettia*, determined by continuous collection, was 6.40% bw/day. However, the rate determined by weight gain was less than half of this, 2.95% bw/day after correction for the control value. There is good reason to believe that the former estimate is the more correct, the latter being reduced by back pressure. Although few data exist, there appears

TABLE IV

Magnesium excretion rates and magnesium permeabilities for some decapod crustaceans in sea water. One dagger indicates that C_m and C_b are magnesium concentrations (mM) in medium and blood, respectively. Two daggers: see text for discussion of the effects of the electrical potential on these values. Three daggers denote the references: (1) Gross and Marshall, 1960; (2) urine and blood [Mg^{2+}]—Riegel and Lockwood, 1961; urine production rate—Binns, 1969a; (3) this report.

Animal (reference)†††	Blood Mg^{2+} mM	Urine Mg^{2+} mM	Medium Mg^{2+} mM	Urine flow rate % bw/day	Mg^{2+} excretion rate $\frac{\mu mol}{g \cdot day}$	Mg^{2+} permeability $\frac{\mu mol}{(C_m - C_b)g \cdot day}$†
Pachygrapsus crassipes (1)	20	305	52.0	3.9	11.9	0.37††
Carcinus maenas (2)	31	250	52 (?)	4.4	11.0	0.52††
Libinia emarginata (3)	44	66	49.0	5.1	3.4	0.68
Pugettia producta (3)	46	70	52.2	6.4	4.5	0.72

to be a relationship between urine production and molt stage since premolt crabs produced urine at greater rates, and postmolt crabs produced urine at lesser rates, than intermolt crabs. The cause of this relationship is a matter for speculation.

The urine production rate was also determined by continuous collection for the osmoconforming spider crab *Libinia emarginata* (5.1% bw/day) and the freshwater crayfish *Pacifastacus leniusculus* (6.0% bw/day). At present, it is difficult to explain, considering water permeabilities and osmotic pressures of blood and media, why *Libinia*, *Pacifostacus* and *Pugettia* produce urine at comparable rates. Regardless, it is clear from many studies that most moderate-sized decapods produce urine at 2 to 10% bw/day when tested at salinities representative of normal habitat salinity, the exception being the freshwater brachyurans which produce no more than about 1% bw/day (Shaw, 1959; Thompson, 1970; Harris, 1975).

The present data suggest that the excretory system of *Pugettia* is responsible for the lowered magnesium concentration in the blood since magnesium is concentrated in the urine, and since blocking the nephropores results in elevated levels of magnesium in the blood. Calcium and potassium are also concentrated in the urine, although not to so great a degree as magnesium. There appears to be some relationship among magnesium, calcium, and potassium in the urine, the concentration of the latter two ions being positively correlated with that of magnesium. By contrast, the calcium concentration in the urine of *Carcinus* is independent of the magnesium concentration (Lockwood and Riegel, 1969).

The concentration of magnesium in the urine of *Pugettia* is negatively correlated with that of sodium. Similar relationships between sodium and magnesium have been reported for *Carcinus* (Webb, 1940; Riegel and Lockwood, 1961), *Pachygrapsus crassipes* (Prosser, Green and Chow, 1955; Gross and Marshall, 1960; Gross and Capen, 1966) and *Cancer magister* (Hunter and Rudy, 1975). Thus, some from of Na^+/Mg^{2+} exchange mechanism may exist. Little is known about the mechanisms of magnesium transport. However, Holliday (1978) has found

that the net magnesium flux in the isolated bladder of *Cancer* is inhibited by ouabain and that only a small part of the opposing net sodium flux is associated with the magnesium flux. In the isolated gut of the insect *Hyalophora cecropia,* magnesium transport is independent of sodium and related, in a complex way, to potassium transport (Wood, Jungreis and Harvey, 1975).

The reabsorption of fluid from the urine, as suggested by the urine/blood (U/B) ratio of filtration markers, is insufficient to account for the U/B ratios of magnesium in a number of decapods (Gross and Capen, 1966; Lockwood and Riegel, 1969; Franklin, Teinsongrusmee and Lockwood, 1978). In *Pugettia,* the U/B ratio of magnesium is about 1.5 and the U/B ratio of inulin may approach this value (Cornell, 1976). However, there is some difficulty in the interpretation of inulin U/B ratios in animals with large bladders (see Riegel, Lockwood, Norfolk, Bulleid and Taylor, 1974; and Cornell, 1976). Thus, it seems unwarranted to conclude that fluid reabsorption accounts for the concentration of magnesium in the urine of *Pugettia.*

Gross and Capen (1966) have shown that the magnesium concentration in the urine of *Pachygrapsus* is a function of the time the urine is held in the bladder. However, during nephropore-occlusion the magnesium concentration in the urine did not increase in *Pugettia,* suggesting that magnesium is concentrated in the antennal gland. A similar experiment on *Pachygrapsus* produced an increase in urine magnesium. In Figure 3 of their report, Gross and Capen (1966) presented results which suggest that the fluid entering the bladders contains 38 mM magnesium, about 20 mM higher than the blood. The urine of *Pugettia* is about 24 mM higher in magnesium than the blood. The implication is that the antennal glands of both animals perform equivalent tasks, and that it is the bladders of *Pachygrapsus* which concentrate most of the magnesium, a function which the bladders of *Pugettia* do not seem to perform.

The concentration of magnesium in the blood must be a function of at least three parameters: the permeability of the body wall, the excretion rate, and the electrochemical potential across the body wall. Neglecting for the moment the electrical potential, and dividing the weight-specific magnesium excretion rate by the concentration difference between the blood and the medium, an estimate of the permeability of the body wall can be obtained. In Table IV these quantities have been computed for four decapods. Taking into account the electrical potential does little to change these results. In *Pugettia* and *Libinia,* the potential is negligible, 0 ± 0.1 mV. Potentials of -2.0 mV (inside negative) for *Pachygrapsus* (Rudy, 1966) and *Carcinus* (Greenaway, 1976) have been reported for animals in SW. This is a small potential and for present purposes may be neglected, however, the permeability of *Pachygrapsus* and *Carcinus* may be slightly less than indicated. The data in Table IV suggest that those crabs which have much reduced the magnesium concentration in their blood have done so by lowering the magnesium permeability and by raising the rate of magnesium excretion.

The significance of magnesium regulation in crustaceans, first noted by Robertson (1949, 1953), is that a strong negative correlation exists between the activity of an animal and the magnesium concentration in its blood. Thus, animals with low magnesium levels tend to be highly active, while those with high levels

tend to be lethargic. Robertson (1953) has pointed out that high extracellular levels of magnesium have been found to block neuromuscular transmission in *Carcinus* (Katz, 1936) and to reduce the mechanical response of isolated crayfish legs to electrical stimulation (Waterman, 1941).

This paper constitutes part of a doctoral dissertation submitted to the Department of Zoology, University of California, Berkeley. It is a pleasure to thank Dr. Ralph Smith for his encouragement and guidance during the course of this work. I am grateful to Dr. Cadet Hand and the laboratory staff for the use of the facilities of the Bodega Marine Laboratory, Bodega Bay, California. I thank Dr. Robert Josephson for the opportunity to pursue my work while acting as a course assistant in the Experimental Invertebrate Zoology Course at the Marine Biological Laboratory, Woods Hole, Massachusetts. Financial assistance in the form of a one-year University Fellowship was greatly appreciated. My wife, Mary, deserves special thanks.

Summary

1. A new technique for the continuous collection of crab urine is described. Estimates of urine production, based on this technique, indicate that specimens of *Pugettia producta* in sea water produce urine at 6.4% body weight (bw)/day. Premolt and postmolt crabs produce 30 and 3.0% bw/day, respectively. Intermolt specimens of *Libinia emarginata* produce 5% bw/day.

2. The urine production rate for specimens of *Pugettia*, estimated by weight gain following 24 hr of nephropore-occlusion, is 3.0% bw/day. This is significantly less than that determined by the continuous collection of urine, suggesting that back pressure can interfere with urine production.

3. Ion regulation was examined in specimens of *Pugettia*. When expressed as a percentage of their concentrations in sea water, the values in blood plasma of chloride, sodium, magnesium, calcium, and potassium are 98, 98, 88, 118, and 105%, respectively, for crabs in sea water. Likewise in the urine, the values for these same ions are 99, 96, 135, 124, and 114%, respectively. Ninhydrin positive substances, measured with glycine standards, are 3.7 and 0.39 mM in blood plasma, respectively. The electrical potential across the body wall of both species of crab is zero.

4. In *Pugettia*, blocking the nephropores causes an increase in the magnesium concentration in the blood, suggesting that the excretory system is mainly responsible for regulating this ion. However, blocking the nephropores causes no change in the magnesium concentration of urine stored in the bladder, which suggests that the antennal gland is mainly responsible for concentrating magnesium in the urine. *Libinia* and *Pugettia* excrete magnesium at a lower rate, and are more permeable to magnesium, than brachyurans which are strong magnesium regulators.

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